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**REMARKS**

Claims 1-8, 16 and 17 are pending.

The invention relates to a method for isolating a pluripotent cell which is at least partially committed to a given developmental pathway, that includes the step of sorting a population of pluripotent cells according to Sox gene expression.

*Priority*

As requested, Applicants are including a certified copy of the Great Britain foreign priority application (UK 9828383.1) filed on December 22, 1998.

*Double Patenting*

The Examiner states that claims 1-8, 16 and 17 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-8 of copending Application No. 09/464,146.

In response to this rejection, Applicants submit that they will submit a terminal disclaimer to disclaim any portion of a patent issuing from the present application which would extend beyond the term of a patent issuing from the 09/464,146 application, upon notification of allowable claims in the present application.

**Rejection of Claims 1-8, 16 and 17 under 35 U.S.C. §112, First Paragraph**

Claims 1-8, 16 and 17 are rejected under 35 U.S.C. §112, first paragraph, for alleged lack of enablement.

*Breadth of the Claims*

The Examiner asserts that "the claims broadly encompass the use of any pluripotent cell derived from any organism whatsoever and any Sox gene. Further, the claims are broadly drawn to the use of sorted cells."

Any pluripotent cell, organism or Sox gene

The Examiner states that "[t]he method broadly encompasses the use of the method in any type of pluripotent cell whether that cell is of hematopoietic origin, neural origin, of ectoderm or endoderm. This breadth is striking and extreme, since the invention presumes that cells intending to develop into any particular tissue type will commit to that lineage using the same set of genetic elements."

Claim 1 has been amended to add the limitation "detecting expression of a Sox gene selected from the group consisting of Sox15, Sox16 and any one of the Sox genes provided in Table 1."

Applicants submit that the specification teaches in Table 1 (p. 14) the Sox gene family as of the filing date of the instant application. As evidenced by this Table, as of the filing date of the instant application, it was known in the art that Sox gene family members are expressed in a wide variety of tissues and cell types including genital ridge, CNS, UGR, lens, PNS, gut, oocytes, lymphocytes, heart, spermatid, brain, testis, pre-cartilage, neural crest, Schwann cells, kidney, lung (including lung endoderm), ovaries, arteries,  $\beta$ -cells, retina, fibroblasts, lymphoblasts, ectoderm and zygote.

The specification also provides GenBank accession numbers for 22 Sox genes at p. 17, lines 3-14 wherein it is stated, "At least the following Sox genes are known; others may be isolated by homology searching. *Sox21* (GenBank Accession No. AF107044); *Sox14* (GenBank Accession No. 107043); *Sox13* (GenBank Accession No. AB104474); *Sox10* (GenBank Accession No. AJ001183); *Sox22* (GenBank Accession No. U35612); *Sox18* (GenBank Accession No. L35032); *Sox11* (GenBank Accession No. U23752); *Sox1* (GenBank Accession No. Y13436); *Sox2* (GenBank Accession No. Z31560 and U12532); *Sox3* (GenBank Accession No. X94125); *Sox4* (GenBank Accession No. X70683); *Sox5* (GenBank Accession No. S83306);

*Sox6* (GenBank Accession No. U32614); *Sox7* (GenBank Accession No. A115903/P40646); *Sox9* (GenBank Accession No. S74504/5/6); *Sox12* (GenBank Accession No. U70442); *Sox13* (GenBank Accession No. AB006329); *Sox15* (GenBank Accession No. AB104474); *Sox16* (GenBank Accession No. L29084); *Sox17* (GenBank Accession No. D49473); *Sox19* (GenBank Accession No. X98368); *Sox22* (GenBank Accession No. U35612)."

Applicants submit that in view of the above, as of the filing date of the instant application, it was known in the art that a variety of cells and tissue types express a Sox gene family member. It was also known in the art as of the filing date of the instant application that members of the family of Sox genes are involved in the development and elaboration of particular cell lineages (see Wegner et al., 1999, Nucleic Acids Res., 27:1409; Pevny et al., 1998, Development, 125: 1967; Nishiguchi et al., 1998, 12: 776). One of skill in the art would recognize that a pluripotent cell which is at least partially committed to a given developmental pathway (for example, genital ridge, CNS, UGR, lens, PNS, gut, oocytes, lymphocytes, heart, spermatid, brain, testis, pre-cartilage, neural crest, Schwann cells, kidney, lung (including lung endoderm), ovaries, arteries,  $\beta$ -cells, retina, fibroblasts, lymphoblasts, ectoderm and zygote) could be isolated by "detecting expression of a Sox gene selected from the group consisting of Sox15, Sox16 and any one of the Sox genes provided in Table 1", as claimed in claimed 1.

In view of the amendment to claim 1, Applicants submit that amended claim 1 is directed to pluripotent cells that differentiate to a tissue or cell type that expresses any of Sox15, Sox16 and any one of the Sox genes provided in Table 1. Applicants submit further that amended claim 1 is directed to any organism that expresses any of Sox15, Sox16 and any one of the Sox genes provided in Table 1. Further, amended claim 1 is directed to expression of a Sox gene selected from the group consisting of Sox15, Sox16 and any one of the Sox genes provided in Table 1.

A pluripotent cell is defined in the instant application at p. 3, lines 15-24, as follows. "As used herein, a "pluripotent cell" is a cell which may be induced to differentiate, *in vivo* or *in vitro*, into at least two different cell types. These cell types may themselves be pluripotent, and capable of differentiating in turn into further cell types, or they may be terminally differentiated,

that is incapable of differentiating beyond their actual state. Pluripotent cells include totipotent cells, which are capable of differentiating along any chosen developmental pathway. For example, embryonal stem cells (Thomson *et al.*, (1998) Science 282:1145-1147) are totipotent stem cells. Pluripotent cells also include other, tissue-specific stem cells, such as neuronal stem cells, neuroectodermal cells, ectodermal cells and endodermal cells, for example gut endodermal cells, and mesodermal stem cells, which have the ability to give muscle or skeletal components, dermal components such as skin or hair, blood cells, etc.”

It is also stated at p. 36, lines 20-25, “Suitable pluripotent precursor cells may be derived from a number of sources. For example, ES cells, such as human ES cells and cells derived from Germ cells (EG cells) may be derived from embryonal tissue and cultured as cell lines (Thomson *et al.*, (1998) Science 282:1145-1147). Alternatively, pluripotent cells may be prepared by retrodifferentiation, by the administration of growth factors or otherwise, or by cloning, such as by nuclear transfer from an adult cell to a pluripotent cell such as an ovum.”

Applicants submit that in view of all of the above, amended claim 1 and dependent claims 2-8, 16 and 17 are properly enabled.

#### Sorted Cells

The Examiner asserts, “[t]he claims are also drawn to a broad use of sorted cells, after detection by some means. Since only two modes are known for detection followed by FACS sorting in the prior art, which rely on either antibody or nucleic acids for the detection, and no additional disclosure is provided in the specification on methods of such sorting, the claims are limited to the prior art methods, which all teach the use of dead cells.”

Applicants respectfully disagree.

The specification teaches in Example 6, and in particular at p. 53, line 22- p. 54, line 5, a method of isolating viable cells by sorting the cells according to Sox gene expression. It is stated at p. 53, line 22-p.54, line 5, “To attempt to isolate the neural progenitor pool, ES cells are used

in which the bifunctional selection marker/reporter gene  $\beta$ geo has been integrated into the *Sox2* gene by homologous recombination. When induced to differentiate as described above, approximately 50% of these cells stain for  $\beta$ -galactosidase activity, consistent with the proportion of cells that express *Sox2* protein. Therefore, application of G418 to the differentiating cultures should eliminate *Sox2*-negative non-neural cells. G418 (200 g/ml) is added after retinoic-acid induction, either during embryoid body culture or upon plating. In both conditions appreciable cell killing is evident. **Crucially, however, large numbers of cells survive that exhibit the small, ovoid morphology typical of neuroepithelial cells.** Over 90% of these cells show prominent  $\beta$ -galactosidase staining. Expression of *Sox1* and *Sox2* proteins is confirmed by immunostaining. Consistent with a neuroepithelial identity, the cells also express nestin.” (Emphasis added) Applicants submit that it was known in the art as of the filing date of the instant application that neural stem cells specifically express nestin (Lendahl et al., 1990, Cell 60:585-595; Dahlstrand et al., 1992, J Cell Sci., 103 ( Pt 2):589).

In view of the above, Applicants submit that the specification clearly teaches a method of sorting cells according to *Sox* gene expression wherein a viable population of cells are isolated.

#### *Quantity of Experimentation*

The Examiner asserts that “[t]he quantity of experimentation in this area is large since there is significant variability in the function and activity of each of the *Sox* genes in each of the cell types and different sources...In order to use any given *Sox* gene, abundant and inventive experimentation would be necessary in order to determine the biological and molecular roles of the molecule.”

Applicants respectfully disagree.

The invention as claimed in claim 1 relates to the use of *Sox* gene expression as a marker for a pluripotent cell which is at least partially committed to a given developmental pathway. The method of the claim requires expression of a *Sox* gene selected from the group consisting of *Sox15*, *Sox16* and any one of the *Sox* genes provided in Table 1. Claim 1 does not require

knowledge of the "biological and molecular roles of the molecule [Sox gene]", as asserted by the Examiner.

*Unpredictability*

The Examiner states, "the art teaches that it is entirely unpredictable what function Sox genes have in cells." The Examiner also states, "[g]iven the unpredictability found in the known Sox genes, it is even more unpredictable what effects as yet unidentified Sox genes in the tens of thousands of different possible cells would have."

Applicants submit that the invention as claimed in amended claim 1 relates to the use of Sox gene expression as a marker for a pluripotent cell which is at least partially committed to a given developmental pathway. The method of amended claim 1 requires expression of a Sox gene selected from the group consisting of Sox15, Sox16 and any one of the Sox genes provided in Table 1. Claim 1 does not require knowledge of the "function Sox genes have in cells", as asserted by the Examiner. Further, amended claim 1 is limited to known Sox genes therefore rendering the issue of unpredictability of as yet unidentified Sox genes moot.

The Examiner also states, "[a] separate area of unpredictability concerns the isolation and sorting of the Sox expressing cells..it is unpredictable how a 'pluripotent' cell can be isolated using a intracellular marker without killing the cell and thereby rendering the cell not 'pluripotent'."

As stated above, the specification teaches in Example 6, and in particular at p. 53, line 22- p. 54, line 5, a method of isolating viable cells by sorting the cells according to Sox gene expression. Example 6 teaches further at p. 54, lines 6-25,

"Accordingly, neural cell types may be isolated by expression of a marker associated with *Sox2*, starting with a population of totipotent cells which has been induced to differentiate *inter alia* into a neural pathway.

In order to determine whether the *Sox2*-selected population have proliferative capacity,  $\beta$ FGF is added to plated cultures. This

results in a major stimulation of cell division. The expanded cells predominantly retain undifferentiated neural morphology and show strong X-gal staining indicative of *Sox2* expression. Such cultures can be amplified and serially passaged for at least three weeks, which is significantly longer than the proliferative phase of neurogenesis in the mouse embryo.

In the absence of mitogen, *Sox2*-selected precursor cells begin to extend neuritic processes within 48 hours and by 96 hours form a network of neuron-like cells. The pan-neuronal markers neurofilament light chain, microtubule-associated proteins, MAP2 and tau, and  $\beta$ -tubulin III are detectable from 48 hours onwards, coincident with down-regulation of *Sox2* expression. By 96 hours, over 90% of cells express neuronal markers, including neurofilament heavy chain and synapsin I. Cells of non-neuronal morphology are rarely apparent, with the exception of the occasional GFAP-positive astrocyte. Astrocyte numbers increase if serum of FGF is added to the cultures. Maturation of the neuronal cells, evidenced by production of gamma-aminobutyric acid (GABA) and glutamate neurotransmitters, and further elongation of neurites with dendritic sprouting is achieved on transfer to Neurobasal medium supplemented with B27 and horse serum."

As recited above, a pluripotent cells, as defined in the instant specification, includes "neural stem cells".

In view of the above, Applicants submit that the specification teaches a method of sorting cells according to Sox gene expression wherein a viable population of pluripotent cells are isolated.

#### *Working Examples*

In response to the Examiner's statement that "[t]he specification has no working examples of isolation of pluripotent cells by detection of Sox gene expression followed by sorting of the cells", Applicants assert that Example 6 teaches a method of sorting cells according to Sox gene expression wherein a viable population of pluripotent cells are isolated.

*Guidance in the Specification*

The Examiner states "[t]he specification...does not teach the sequence of Sox genes from even a representative fraction of all of the different organisms which are included in the scope of the claim, nor are a representative number of precursor cell types given."

Claim 1 has been amended to add the limitation of "detecting expression of a Sox gene selected from the group consisting of Sox15, Sox16 and any one of the Sox genes provided in Table 1". The number of Sox genes included in the Markush group of amended claim 1 is 28. The specification provides GenBank accession numbers for 22 of these 28 Sox genes at p. 17, lines 3-14 (recited above).

Applicants are of the opinion that the sequence of 22 of 28 Sox genes is a representative fraction.

The Examiner also states, "[w]hile the specification does have a table...which discusses 24 Sox genes and lists a few different species in which these genes are found, in only a very few of these situations is any biological role known."

Applicants submit that, as discussed above, amended claim 1 does not require knowledge of the biological role of the Sox genes of claim 1.

The Examiner also states, "the specification provides no disclosure of how to isolate living pluripotent cells". Applicants submits that as stated above, Example 6 teaches a method of sorting cells according to Sox gene expression wherein a viable population of pluripotent cells are isolated.

In view of all of the above, Applicants submit that claims 1-8 and 16 and 17 are properly enabled and respectfully request withdrawal of the 35 U.S.C. § 112, first paragraph rejection of claims 1-8, 16 and 17.

Rejection of Claims 1-8 and 16 under 35 U.S.C. §112, First Paragraph